Surgical Implantation of Transmitters into Fish

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Abstract

Although the Animal Welfare Act does not cover poikilotherms, individual institutions and policies and legal requirements other than the Animal Welfare Act (e.g., the US Public Health Service and the Interagency Research Animal Committee's Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training) require the review of projects involving fish by institutional animal care and use committees (IACUCs). IACUCs may, however, lack the knowledge and experience to evaluate fish projects judiciously, especially when the projects are in field settings. Surgeries involving implantation of transmitters and other instruments into the coelom, which now comprise a very common research tool in the study of free-ranging fishes, are examples of surgeries that use a broad spectrum of surgical and anesthetic techniques, some of which would not be considered acceptable for similar work on mammals. IACUCs should apply the standards they would expect to be used for surgeries on homeotherms to surgeries on fish. Surgeons should be carefully trained and experienced. Surgical instruments and transmitters should be sterile. Regulations and laws on the use of drugs in animals should be followed, particularly those concerned with anesthetics and antibiotics used on free-ranging fish. Exceptions to surgical procedures should be made only when circumstances are extreme enough to warrant the use of less than optimal procedures.

Key Words: anesthesia; Animal Welfare Act; fish; IACUC; implantation; surgery; telemetry; transmitter

Introduction

The Animal Welfare Act covers homeothermic animals, which would, by definition, exempt fish. Individual institutions and policies and legal requirements other than the Animal Welfare Act, such as those promulgated by the US Public Health Service and the Interagency Research Animal Committee's Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, cause projects involving fish to be reviewed by institutional animal care and use committees (IACUCs[1]). Fish are used extensively as experimental animals at some institutions; however, many IACUC members may lack the knowledge and experience to evaluate fish projects judiciously, especially if the projects are in field settings.

Surgical implantation of transmitters and other instruments into the coelom has become a very common research tool in the study of free-ranging fishes. Such surgeries are performed using a broad spectrum of surgical and anesthetic techniques, some of which would not be considered acceptable for similar work on mammals. Because there are no written standards for surgery and anesthesia on free-ranging fish, IACUCs have little basis to judge the adequacy of proposals involving these techniques. There is a large body of published research documenting problems experienced with fish implanted with transmitters. That experience, when combined with standards of care used on all other vertebrate animal groups, can be used to make recommendations for the performance of anesthesia and surgery on fish in a field setting.

The attachment of transmitters or other instruments to fish is the surgery most commonly performed on free-ranging fish. Telemetry, using either ultrasonic or very high frequency radio transmitters, is an essential tool for the study of such populations. Transmitters are attached to individual fish, but data acquired from following the movements and behaviors of the individual animals are usually extrapolated to the larger population. If the act of placing the instrument in or on the animal causes a lasting iatrogenic alteration in movement or behavior, the data gained from following that animal will be flawed and the conclusions derived from the application of flawed data to the population will be erroneous.

Surgery performed on free-ranging animals is unique in that the patient is rarely observed after surgery is completed, and the patient is released back into the wild. Complications such as altered behavior, altered buoyancy, infection, dehiscence of the incision, and hemorrhage are therefore difficult to observe and are rarely reported. If the implanted transmitter is equipped with a "mortality switch," then a change in signal characteristic occurs when the transmitter does not

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[1]Abbreviations used in this article: AMDUCA, Animal Medicinal Drug Use Clarification Act; FDA, Food and Drug Administration; IACUC, institutional animal care and use committee; MS-222, tricaine methanesulfonate.

move for a set period and the death of the implanted fish is assumed. Because death of an implanted fish is the most easily detected postsurgical complication of transmitter implantation, survival has become the most commonly used criterion of the quality and success of the surgical procedure.

Although death of the implanted individual due to transmitter implantation is a reliable indicator of an adverse effect, it should not be assumed that the absence of death indicates an absence of effect. In addition, an implanted fish may die from causes not related to the implantation procedure. From the viewpoint of the goals of any telemetry project, the loss of signal from an individual that died would be preferable to the acquisition of data from an animal suffering sublethal effects that caused abnormal behavior.

Even recently published books on wildlife telemetry do not describe in any detail the techniques and materials to perform implant surgeries in fish or other vertebrates (Kenward 2001; Millspaugh and Marzluff 2001). Such books go into great detail regarding how to process and interpret telemetry data, but the literature does not include guidelines for the performance of the surgical implantation of the transmitters that produce the data. Perhaps the best analysis of procedures of fish anesthesia and surgery for implanting transmitters is that of Baras et al. (2003), and a thorough review of basic fish anesthesia and surgery is available (Summerfelt and Smith 1990).

Nevertheless, these and other guides do not adopt the standards deemed necessary for surgery on all other vertebrates. The use of aseptic procedures and sterilized equipment, which is standard procedure in avian and mammalian surgery, is not standard in surgeries for the implantation of transmitters into fish. Guidelines for the use of fishes in field research published by professional societies thoroughly admonish IACUCs regarding their duties and limitations in reviewing study plans and proposals involving fish (ASIH/AFS/AIFB 1988). However, the guidelines only mention that implantable transmitters should have a biocompatible coating and that a transmitter with a percutaneous antenna may need to be anchored internally. No guidance is provided on the minimal standards for anesthesia and surgery.

The purpose of this article is to discuss the present state of the surgical implantation of transmitters into the coeloms of fish, and to propose standards for performing such surgeries. The comments in this article particularly concern surgeries performed in field situations, but the principles apply to surgeries anywhere. Although intended as a guide for IACUCs in their review of proposals, this article should also serve the needs of the scientist planning a project involving implantation of transmitters into fish.

Need for Improvements in Anesthesia and Surgery Techniques

The techniques used for transmitter implantations should be improved for the following reasons: (1) Sublethal effects of surgery and anesthesia may affect the quality of data collected and the validity of the conclusions drawn from those data; and (2) there is an increasing moral imperative to treat fish in the same manner as higher level animals. There are few valid reasons not to improve the quality of anesthesia and surgery for transmitter implantation.

As long as the sole criterion for judging the success of an implantation project remains the "black or white" test of survival versus death, the quality of the data gathered must be considered questionable. In many field situations, it may be very difficult or even impossible to measure the sublethal effects of surgery. It is clear that sublethal effects do occur and that they have the potential for invalidating, or at least clouding, the usefulness of the data collected. Many sublethal effects have been studied in both field and laboratory settings. Potential side effects include, but are not limited to, the following: decreased survival (Manns and Whiteside 1979); altered behavior (Diana 1980; Manns and Whiteside 1979; Mesing and Wicker 1986; Thoreau and Baras 1997); loss of buoyancy (Gallepp and Magnuson 1972); loss of equilibrium (Thoreau and Baras 1997); infection (Paukert et al. 2001); dehiscence of the closure as well as loss of transmitters (Baras and Westerloppe 1999; Marty and Summerfelt 1986); passive or active downstream movement after release (Haynes and Gray 1979); decreased feeding (Lucas 1989; Mortensen 1990; Thoreau and Baras 1997); increased predation (Adams et al. 1998b); loss of social standing (Zimmermann 1980; Zimmermann and Bercy 1981); loss of swimming capacity (Arnold and Holford 1978; McCleave and Stred 1975; Mellas and Haynes 1985; Moore et al. 1990); and decreased growth (Baras et al. 2003; Knights and Lasee 1996; Paukert et al. 2001). As Baras et al. (2003) state, ". . . most adverse effects from surgical tagging on fish behaviour and physiology could be alleviated or suppressed by tailoring the surgery procedure, equipment and operative care." Because it may be very difficult to evaluate or measure sublethal effects of capture, handling, anesthesia, and surgery on free-ranging fish, it behooves researchers to use the best possible techniques to minimize adverse effects.

Choice of Transmitter Attachment Method

The least invasive method that provides the desired signal duration and quality should be used to attach a transmitter to an animal. Externally mounted transmitters have often been considered less invasive than surgically implanting the transmitter in the coelom. However, external attachment methods may have longer adverse effects (e.g., infection, entanglement, increased drag, asymmetric drag, increased predation) on the host than internally implanting the transmitter. For example, sutures, plastic bands, or wires placed through tissue to hold an external transmitter on an animal may cause chronic, nonhealing wounds. Transmitters mounted externally on fish may affect balance, increase drag, increase susceptibility to predators, or become entangled in aquatic vegetation (Thorstad et al. 2001). A properly sutured surgical incision heals rapidly and may cause less chronic stress. Most externally mounted transmitters will eventually be shed from the fish, which is usually regarded as an advantage. Implanted transmitters may be shed but should be considered as being present for the lifetime of the fish.

Field Conditions and Condition of Fish

Water temperature is an important variable that influences both surgery and anesthesia. Quinaldine was an effective anesthetic for grass carp (*Ctenopharyngodon idella*) at water temperatures <26°C, but it was lethal at the concentration required to induce and maintain anesthesia at water temperatures >29°C (Schramm and Black 1984). Tricaine methanesulfonate (MS-222[1]) was a suitable anesthetic for grass carp at all water temperatures (Schramm and Black 1984). All brown trout (*Salmo trutta*) implanted when the water temperature exceeded 18°C died within hours of surgery (Clapp et al. 1990).

Probably no single part of the entire transmitter implantation project is as stressful to the fish as the capture event itself. Thanks to the popularity of angling and especially due to the emergence of "catch and release" angling competitions, there have been many studies on the effects of capture on marine and freshwater fish (Bendock and Alexandersdottir 1993; Brick and Cech 2002; Cho et al. 2002; Clements et al. 2002; Cooke et al. 2001; Dunmall et al. 2001; Dwyer et al. 2001; Fletcher 1987; Gallman et al. 1999; Gjernes et al. 1993; Heath and Pritchard 1962; Lowe and Wells 1996; Muoneke 1992; Nuhfer and Alexander 1992; Pankhurst and Dedual 1994; Pepperell and Davis 1999; Schisler and Bergersen 1996; Schreer et al. 2001; Thompson et al. 1997; Tomasso et al. 1996; VanderKooi et al. 2001; Wilde et al. 2000; Wood 1991). Because of the vast numbers of species and habitats of fish, which require a wide array of capture techniques, it is impossible to generate a single protocol for the capture and handling events during transmitter implant projects. In general, fish should be caught with as little stress placed on them as possible, handled as little and as gently as possible, and released as soon as possible.

Reports from some studies indicate that instrumented fish appear reluctant to move immediately after release, either staying near the release site or moving passively with water currents (Groot et al. 1975; Haynes and Gray 1979; Madison et al. 1972; Stasko et al. 1973; Thorstad et al. 2001). Passivity of fish may last from minutes to days, but it has been poorly described as a potential complication of telemetry projects. It is generally ascribed to the cumulative stress of handling and surgery.

Selection of Transmitter Type and Size

The appropriate size and shape of the transmitter to be implanted into the coelom of a fish varies according to the species and size of the fish as well as the purpose of the study, which determines required data storage, transmitter capability, and associated battery size. This subject requires much more research than presently exists to establish a guideline based on scientific evidence. The smallest and lightest transmitter that provides the desired signal type, strength, and duration should be used. Because of miniaturization of electrical components, batteries represent the bulk of the weight of most units. Transmitter weight, as a percentage of the fish's body weight, is the primary limitation on the transmitter that can be surgically implanted. A commonly cited recommendation (Winter 1983) is to limit the weight of internal transmitters to ≤2% of the fish's body weight in air (≤1.25% of the weight in water); however, no data were presented to defend that limit. Other researchers have found that transmitters ranging from 6 to 12% of body weight in air did not adversely affect the swimming performance of rainbow trout (Brown et al. 1999). A preferred index would be the weight of the transmitter in water because tags can weigh the same in air but have different buoyancies in water (Brown et al. 1999; Perry et al. 2001).

The dimensions of the transmitter are also important variables, especially with laterally compressed fish such as bluegills (*Lepomis macrochirus*), or crappies (*Pomoxis* spp.) in which a laterally compressed transmitter might be more suitable than a round transmitter. Larger tags, by both weight and dimension, were found to result in greater mortality (20%) and transmitter loss than small transmitters (0%) implanted into bluegills (Paukert et al. 2001). Growth was retarded for 2 wk, and body condition decreased more in fish implanted with a transmitter that averaged 2.2% of fish body weight than in fish implanted with a transmitter that averaged 1.0% of fish body weight (Paukert et al. 2001).

Transmitters should be as close to neutral buoyancy as possible. Although physostomatous fish may regain buoyancy quickly by ingesting air into their swim bladders, physoclistous fish may sink after implantation of a transmitter. In that event, hours or even days may be required to produce enough gas into their swim bladder to offset the weight of the transmitter (Gallepp and Magnuson 1972; Thoreau and Baras 1997).

If implanted transmitters have percutaneous antennas, the length of the antenna protruding from the body should be stated in any description of the technique. Very long antennas may interfere with swimming ability and may increase the likelihood of predation. Transmitters with antennas that were two to three times the length of the fish were implanted into juvenile chinook salmon (*Oncorhynchus tshawytscha*), but the antennas became entangled and the transmitters were pulled out of the fish (Adams et al. 1998a,b).

Training of the Surgeon

Only personnel with considerable experience in fish anesthesia and surgery should perform surgical implantation of transmitters into fish. Biologists desiring to perform implantation surgeries at the highest possible level of expertise should seek the assistance of a veterinarian experienced in fish anesthesia and possibly request training and advice in anesthetic monitoring, aseptic surgical techniques, and closure methods. Biologists should strive to perform the surgeries with the same level of quality as a veterinarian experienced in the technique would maintain.

Sterilization of Transmitters

The surgical implantation of a nonsterile transmitter into any animal is an inhumane act, which should not be performed. Fish, like mammals, are susceptible to infections from contaminated implants.

Techniques

Dipping or soaking transmitters into solutions of chlorhexidine, isopropyl or ethyl alcohol, benzalkonium chloride, or other such disinfectants does not result in sterilization, although use of the solutions has been documented as acceptable (Burger et al. 1994). In addition, disinfectants are intended primarily for external use, and residual amounts of the chemicals on the transmitter can cause tissue damage or toxicity. Many researchers do not know the principles of sterilization and may believe that soaking in, or a very short exposure of a transmitter to, a solution of disinfectant actually sterilizes it (Bidgood 1980). Because critical restrictions exist, in addition to the necessity of avoiding damage to the instrument, some investigators may believe that attaining sterilization of their transmitters is beyond their logistical capabilities. Because survival of the implanted fish is often the sole criterion of success or lack of adverse effect, there is a failure to question technical methods critically, as long as the implanted fish live.

The techniques for sterilization of implantable transmitters have been poorly covered in telemetry literature (Burger et al. 1994). Sterilization denotes the complete elimination of all life forms, including spores, parasite eggs, and cysts. Disinfection eliminates most, but not all, microorganisms. Disinfectants vary in their chemical makeup, and some are superior to others. Important variables in the use of disinfectants include chemical composition, concentration, contact time, temperature, hardness of the water used as a diluent, and presence of organic material. Autoclaving—attaining a temperature of 121°C for 15 min at 15 psi in a pressure vessel—is the most commonly available laboratory method of sterilization. However, the temperature and pressure required make autoclaving unsuitable for sterilizing electrical devices, especially those with coatings of wax or plastic.

Two techniques are most suitable for sterilization of implantable transmitters: exposure to ethylene oxide gas, and immersion for an adequate period in a true sterilizing solution such as glutaraldehyde. Although both techniques have disadvantages (described below), any alternative methods are less available or less suitable. Ethylene oxide sterilization has the advantage of allowing prepackaging of transmitters in envelopes that can maintain sterility of the transmitter during transportation into the field. The use of an immersion solution is most typically performed in the field just before surgery.

Exposure to Ethylene Oxide Gas

Ethylene oxide gas sterilization requires installation of a sterilizing box and a ventilation system. The gas is extremely toxic and carcinogenic, and human exposure during processing must be monitored with exposure badges. Transmitters must be packaged in materials that are permeable to the sterilizing gas yet sufficient to prevent contamination of the transmitters when they are removed from the sterilizer. A typical gas sterilization cycle takes approximately 12 hr to complete, and the sterilized transmitters should be allowed to air freely for an additional 12 to 24 hr before use to ensure the dissipation of all absorbed ethylene oxide. Any material surrounding the transmitter, such as the tape used to hold a magnet in position, must be permeable to the sterilization gas. Manufacturers typically supply transmitters with plastic tape used to hold the magnets in place. This tape must be removed and replaced with a porous paper tape.

An advantage of using ethylene oxide is that the sterile transmitters can be transported to the field and used immediately. No delays for treatment are required as with solutions of disinfectant, and it is not necessary to rinse the residual disinfectant before implantation.

Immersion in a Sterilizing Solution

Liquid ("cold") sterilants, such as glutaraldehyde, require total immersion of the transmitter in a liquid for a period of hours. Careful adherence to label instructions for the product is necessary to ensure sterilization. Generally, transmitters manufactured for implantation are waterproof and can survive immersion. In fact, one of the advantages of using a liquid sterilant is that transmitters with defective waterproofing will be revealed by the failure of the unit in the solution, which prevents the implantation of a transmitter that is likely to fail. Cold sterilants have a defined shelf life and must be used before their expiration date to ensure effectiveness. Besides limited shelf life, these solutions typically require the mixing of two components for activation. Once mixed, the solution has a very limited active period. The temperature of the solution and the duration of contact are important variables and are defined in the user instructions for the solution.

Not surprisingly, cold sterilants represent a risk for human exposure. Such solutions produce noxious fumes and may be used only in areas with good ventilation. Protective equipment, including safety glasses and gloves, must be worn during periods of exposure. These solutions are classified as hazardous materials for the purposes of shipping, and pertinent laws must be followed. By their nature, liquid disinfectants must be used just before surgery. Adequate time must be allowed for the transmitters to remain in the solution, and care must be taken to ensure that all parts of the transmitter, including antenna, are immersed. If additional transmitters are added to the sterilizing solution after the sterilizing period has begun, contamination has occurred and the timing of the sterilizing period must begin again. Similarly, any handling of the transmitters while they are in the sterilizing solution (e.g., to remove a magnet to start a transmission duty cycle) must be performed while wearing sterile gloves and using aseptic technique.

After the surgical incision is made, the transmitter is removed from the solution, thoroughly rinsed with sterile saline or water, and immediately implanted. The sterilizing solution must also be washed free from the gloves of the surgeon who removed the transmitter. Because of the need to rinse off all traces of the solution thoroughly, this method of sterilization is not suitable for a transmitter that has any absorbent materials attached.

Once removed from the solution, the transmitter cannot be allowed to touch any nonsterile surface. In theory, it is possible to sterilize transmitters with chemical solutions before leaving for the field, but the transmitters must be thoroughly rinsed with sterile saline, dried with sterile towels, and then placed into presterilized packaging before transportation. In practice, this approach to the problem can be clumsy, and it is not often performed.

Sterilization of Surgical Instruments

The use of nonsterile surgical instruments for the implantation of transmitters is an inhumane act, which should not be performed. Most of the comments above pertaining to sterilization of transmitters also apply to the sterilization of instruments used for surgery. A separate set of sterile surgical instruments should be used for each implantation surgery. If instruments are to be used again, they must be cleaned first and then autoclaved or held in a sterilizing (not disinfecting) solution for an adequate period of time and at a temperature sufficient to permit sterilization.

The presence of organic debris such as blood or tissue fluid on the instruments interferes with chemical sterilization. Separate containers must be used to avoid cross-contamination. The instruments must be rinsed thoroughly before use and protected from recontamination. It is possible to purchase autoclaves that are suitable for use in a field setting when electricity is available. A pressure cooker can also be used to sterilize instruments, and it has the advantage of not requiring electricity.

Because inexpensive surgical instruments are available, the preferred method is to purchase sufficient complete sets of surgical instruments to package and sterilize (preferably by autoclaving) a set of instruments for each surgery to be performed. A calculation of the costs of travel to the field site, accommodations, capture of the animals, field equipment such as nets and boats, salaries and benefits for the personnel involved, and purchase of the transmitters and receivers will reveal that the cost of a sufficient number of sets of surgical instruments is a small proportion of the total cost of the project. In addition, the surgical instruments can be reused, so that costs may be amortized over years of projects. Finally, the cost of the instruments is trivial when one considers the quality and reliability of data acquired when surgery is performed using properly sterilized instruments.

Preparation of the Surgery Area

The physical environment in which the implantation surgeries are performed is an important component, which determines the quality of the surgery. Several anesthesia and surgical systems for use with fish have been described (Brown 1999; Courtois 1981; Goetz et al. 1977; Lewbart and Harms 1999; Reinecker and Ruddell 1974; Smith and Bell 1967; Summerfelt and Smith 1990). In practice, these devices tend to be bulky and are perhaps most suited for use in a laboratory.

Regardless of the physical setup used for surgery, all surfaces should be made of materials impermeable to water, to facilitate disinfection and to reduce the chance of transferring pathogens. At a minimum, a clean surface with good lighting is preferred. Fish are typically anesthetized in buckets or tanks of anesthetic-containing water and then placed in dorsal recumbency in a V-shaped trough. Water with anesthetic (usually at a concentration lower than the inducing solution) is circulated across the gills intermittently (e.g., using a kitchen baster) or continuously (using small pumps) to maintain a surgical plane of anesthesia.

The surgical area should be clean and should be wiped down with a solution of disinfectant before placing a fish on it. At a minimum, the V-shaped trough should be rinsed with clean water between fish. To minimize the chances for transfer of pathogens between fish populations, all equipment used for the capture, holding, anesthesia, surgery, recovery, and movement of fish during a transmitter implantation project should be thoroughly cleaned and then thoroughly disinfected before use with a different fish population.

Anesthesia and Analgesia, Including Compliance with Drug Laws

The surgical implantation of a transmitter into the coelom of a fish without anesthesia is an inhumane act, which should not be performed. Fish surgeries that have been performed without any anesthetic have relied on low water and air temperatures to reduce pain (Filipek 1989). However, any procedure that causes more than "momentary pain or discomfort" should be performed using a general anesthetic that provides adequate analgesia (relief from pain). Subcutaneous implantations can be performed with injection of a local anesthetic. For more invasive procedures, a general anesthetic must be used.

No formal definition of "momentary pain or discomfort" has been made for any species. It is logical that anything more invasive than a needle stick and any restraint more than brief holding exceeds "momentary pain or discomfort." Fortunately, several immersion anesthetics are available and well developed for use in fish, although their use is restricted in fish intended for immediate release into waters from which they might be caught and consumed by humans.

Compliance with drug laws presents a dilemma for scientists who wish to implant fish and release them into natural waters in the United States. Current drug laws tightly regulate the use of chemicals and drugs in animals that have any chance of entering the human food chain. The Animal Medicinal Drug Use Clarification Act (AMDUCA[1]) of 1994 (PL 103-396 1994), which took effect in 1996 (AMDUCA Rules and Regulations 1996), made it legal to use drugs approved for use in one animal in a different species. The US Food and Drug Administration (FDA[1]) enforces the act. Compliance with AMDUCA requires involvement of a veterinarian within the context of a valid veterinarian-client-patient relationship, and retention and availability of certain records for 2 yr for possible FDA inspection. Because AMDUCA is a new law and its interpretation is still evolving, fishery biologists should seek the advice of a veterinarian knowledgeable about the requirements of this law.

The FDA has approved only one anesthetic, MS-222, for use in fish. Only the two approved forms of the drug (Finquel®, Argent Chemical Laboratories, Redmond, WA; Tricaine-S®, Western Chemical, Ferndale, WA) can be used as an anesthetic legally. The MS-222 that is marketed by chemical supply companies is not approved for use as an anesthetic drug, and such use is illegal. Because an FDA-approved anesthetic exists for use in fish, it is technically illegal under AMDUCA to use any other anesthetic without good justification.

Clove oil (with the active ingredient eugenol) is frequently used for anesthesia of fish that will be released into waters where there is the chance they will be caught and eaten by humans. There may be interspecific differences in the reaction of fish to clove oil (Griffiths 2001). Clove oil is listed as "generally regarded as safe" as a food additive by the FDA; however, because its use as a fish anesthetic is not considered by FDA as a food use, its use is illegal (FDA 2002). At the time of this writing, in the strict legal sense, only CO_2 should be used as an anesthetic for fish that will be immediately returned to the wild. Although CO_2 has not been approved, FDA has stated that its use as a fish anesthetic is of low regulatory interest (FDA 2002). Other than CO_2, there is no anesthetic drug that can be used legally without a withdrawal period for fish being returned to the wild.

The FDA requires a 21-day withdrawal period for MS-222-exposed fish, which is problematic for projects that require immediate return of anesthetized fish to waters from which they might be caught for consumption by humans. Solutions of MS-222 in fresh water are acidic, but in seawater are basic (Ohr 1976). Increasing concentrations of MS-222 cause concomitantly more acidic pH levels. An unbuffered solution of MS-222 >1.75 g/L in distilled water has a pH of <3.0 (Ohr 1976). Unbuffered MS-222 lowers the pH and bicarbonate alkalinity of the water, thereby increasing pCO_2 and conductivity (Smit and Hattingh 1979). Unbuffered MS-222 causes acidemia, increased blood urea nitrogen concentrations, hypercholesterolemia, and an increase in ACTH production (Soivio et al. 1977, Wedemeyer 1970). When MS-222 is used, it must be buffered with alkali to between pH 7 and 8. This task is most easily performed by supersaturating the routine 10 g/L stock solution with sodium bicarbonate. Buffering MS-222 prevents changes in water quality, which results in lowered induction times, increased concentration of MS-222 in the bloodstream, and increased recovery times (Smit and Hattingh 1979). MS-222 causes an increased hematocrit in anesthetized fish, an effect that is mostly due to erythrocyte swelling (an increase in mean cell volume), although an absolute increase in erythrocyte numbers in circulation occurs with higher concentrations (Ryan 1992).

One protocol for transmitter implantation recommended that the decrease in pH caused by MS-222 could be resolved by first anesthetizing two or three fish in the anesthetic solution to cause a biological buffering effect before using the solution on fish intended for surgical implantation (Bidgood 1980). This is an example of an improper procedure that is passed around as a standard of care. Besides ignoring the welfare of the fish used to change the initial pH of the solution, it is unnecessary, considering the ease of chemically buffering the solution.

Anesthetics themselves may have deleterious effects. MS-222 reportedly causes ultrastructural damage to the olfactory epithelium of channel catfish (Lewis et al. 1985). However, no deleterious effect on chemosensory behavior (homing instinct) of Pacific salmon anesthetized with MS-222 could be detected (Quinn et al. 1988). MS-222 caused changes in the hematocrit, hemoglobin, lactate, glucose, and electrolyte levels of blood that persisted from a few hours to 24 hr (Soivio et al. 1977). Unbuffered MS-222 caused a greater degree of change in blood parameters than buffered MS-222.

Preparation of the Surgical Field and Surgeon

Some authors advise against the use of disinfectants to prepare the skin for incision (Stoskopf 1995). Commonly used disinfectants may cause damage to fish skin (Nemetz and MacMillan 1988), and some (e.g., alcohol) should not be used at all on fish (Briggs 1997). Disinfectants containing high concentrations of alcohol are astringent and should not be used. Povidone iodine and chlorhexidine diacetate are disinfectants that can be used on most fish species if diluted. A 10% povidone-iodine detergent solution (0.75% free iodine) was applied to the surgical sites of rainbow trout (*Oncorhynchus mykiss*), followed by a 10% povidone-iodine solution (without detergent; 1% free iodine) by wiping the site with a disinfectant-soaked cotton swab in the direction of scale growth (Wagner et al. 1999). Compared with paired incision sites, there were no differences in the rate of healing or the histological response; and there were no systemic bacterial infections. Benzalkonium chloride, however, caused irritation of the skin of flathead catfish (*Pylodictis olivaris*) when used as a disinfectant (Hart and Summerfelt 1975).

The production of mucus may be so profuse in some species that the disinfection of the skin becomes moot. If a disinfectant is used, it should be diluted and applied by gently daubing the skin with gauze or a cotton swab soaked in the disinfectant. Vigorous rubbing or scrubbing of the skin is unnecessary and may cause injury. Indeed, an exuberant and unwanted response of the mucous glands may result from excessive scrubbing of the surgery site. Application of the disinfectant should be restricted to the location of the incision and the antenna exit site (if any). Care should be taken to avoid inadvertent exposure of the fish's gills to disinfectant, and to avoid contamination of the water used to irrigate the gills.

The surgeon should wear sterile surgical gloves and change gloves between surgeries to maintain the highest level of asepsis possible. Although a full presurgical hand scrub is not practical in field situations, the surgeon should at least wash hands before doing surgery. Masks and head coverings are recommended but are not essential.

Surgery, Including Selection of Suture and Suture Pattern

The surgical approach chosen for implantation of transmitters may be an important factor, although it has not been studied thoroughly. Schramm and Black (1984) reported their preference for a midline ventral incision compared with a lateral incision for insertion of transmitters. Approximately 75% of brown trout implanted using a lateral approach to the coelom died, whereas only one fish implanted through a ventral incision died (Clapp et al. 1990). Because of the thicker, more vascular anatomy of the lateral body wall, a ventral approach to entry of the coelom should be used unless another approach can be justified.

The failure to close an incision made into the coelom of an aquatic animal is an inhumane act, which should not be performed. All incisions should be closed using sutures or surgical staples. Even if mortality is determined to be low when incisions are not closed, morbidity can still occur that may affect the quality of the telemetry results. The shortest possible incision should be made that allows insertion of the transmitter into the coelom without excessive pressure. Incisions 5 to 7 cm in length were made in channel catfish (*Ictalurus punctatus*) but were not surgically closed, and mortality was very low (Carmichael 1991). However, the fish were treated twice daily for 10 days with nitrofurazone and salt, a protocol that is impractical for most field studies.

Monofilament suture should be used to reduce wicking of water into the coelom. Monofilament, absorbable, polyglyconate suture produced less inflammation in the skin of koi after 7 and 14 days compared with other types of suture material (Hurty et al. 2002). Absorbable monofilament suture that was used to close surgical incisions in largemouth bass (*Micropterus salmoides*) lasted longer and provided faster healing (3 wk) than absorbable braided or chromic gut suture (Gilliland 1994).

The use of braided silk suture should be avoided. Braided silk suture used in blue tilapia (*Oreochromis aureus*) slowed healing (Thoreau and Baras 1997) and caused the most reaction in the skin of koi (*Cyprinus carpio*). Braided silk suture and a vertical mattress suture pattern (despite suture type) caused significantly more inflammation than absorbable or nonabsorbable monofilament suture material in a simple interrupted pattern in adult rainbow trout (Wagner et al. 2000). However, the use of a horizontal mattress pattern in lampreys (*Petromyzon marinus*) resulted in a lower rate of incisional dehiscence, improved apposition of wound edges, and longer persistence of the sutures compared with a simple interrupted pattern or with cyanoacrylate adhesive (Lowartz et al. 1999).

A single-layer, simple interrupted suture pattern is commonly used for the closure of most surgical incisions in fish. A continuous suture pattern may be preferable for the following reasons: it leaves fewer knots and suture ends to cause irritation, it affords less surface area on which bacteria can absorb, and it is faster to deploy once skills are acquired. A two-layer closure may add to the security of the incision, especially in thick-walled fish; however, experimental data on this technique are lacking. The presence of a transmitter in the coelom can increase the pressure on the incision site and cause inflammation, which contributes to dehiscence of the incision and loss of the transmitter (Kaseloo et al. 1992; Schramm and Black 1984; Wagner and Stevens 2000). If possible, the transmitter should be placed in the coelom in a manner that avoids direct contact with the incision.

Surgical staples are an option for closing the surgical incision (Filipek 1989; Mortensen 1990; Mulford 1999). However, it is important to select the proper size staples and to perform the stapling properly to ensure apposition of the edges of the incision. Some investigators have tried staple closures but have returned to suturing because they believe that stapling increased the mortality rate or transmitter loss rate (Haeseker et al. 1996; Starr et al. 2000). Incisions have been closed using medical cyanoacrylate glue (Nemetz and MacMillan 1988). However, cyanoacrylate glue loses its effectiveness when it is constantly immersed in water (particularly seawater), which might result in trans-incisional loss of the implanted transmitter. Dehiscence of incisions and evisceration of lampreys (*Petromyzon marinus*) occurred at a higher rate when incisions in lamprey were closed with cyanoacrylate compared with suture closures; and gross inflammation was present after 2 wk in 60% of the glued fish compared with only 20% of the sutured fish (Lowartz et al. 1999). Approximately 70% of black crappies (*Pomoxis nigromaculatus*) lost their transmitters through dehiscence of incisions closed with tissue glue (Petering and Johnson 1991).

Temperature may be an important factor in determining the rate of healing of incisions made in fish. Fish living at cold temperatures typically heal at a slower rate, sometimes requiring many weeks (Knights and Lasee 1996, Ross 1982). Both the immune response and the inflammatory response of fish are influenced by temperature. Healing of experimental wounds in a tropical and a temperate water species was directly related to temperature (Anderson and Roberts 1975).

Use of Antibiotics

Antibiotics should not be used as a substitute for aseptic technique. Careful adherence to procedure with due attention to the prevention of contamination of incision, transmitter, or instruments obviates the perioperative use of antibiotics. The use of a single dose of an antibiotic is of little or no advantage. The value of antibiotic use in surgery on mammals may vary with the type of surgery use of antibiotics and is still controversial (Whittem et al. 1999). To be of greatest value, antibiotics must be administered before the incision is made. No systematic study results of the value of perioperative administration of antibiotics in fish transmitter implantation surgeries have been published.

Tetracycline is an antibiotic that is often injected into the coelom of fish implanted with transmitters. The origin and intent of this practice are obscure because tetracycline lacks a broad spectrum of activity against bacteria that might cause tissue infections. Its continued use is also puzzling because much better antibiotics have been developed. Tetracycline has been approved for use in fish by the FDA only as a food additive in salmonid fish and catfish, with a 21-day withdrawal period. Its direct application during transmitter implantation surgery constitutes extra-label drug use and must meet the requirements of AMDUCA. Some biologists believe that tetracycline reduces the amount of tissue adhesion to the transmitter, although to date there is no documentation to that effect. Reduction of adhesions to the transmitter might be useful in some species, which tend to shed transmitters by their absorption into the intestinal lumen with subsequent elimination via the anus. However, especially for transmitters equipped with a percutaneous antenna, adhesions may help to stabilize the transmitter in the coelom and would therefore be a desirable state. It is also possible that the use of tetracycline was introduced originally as a means of fluorescent marking of fish bones for future identification of treated fish, and its continued use is the result of a belief that it helps to prevent infection after implantation of a transmitter.

Other chemicals have been used in some studies to reduce contamination. Hinch et al. (1996) applied malachite green or table salt (for free-ranging fish) to sutures in an attempt to prevent fungal infections, although there was no documentation that such infections occurred or that chemical treatment of the suture material prevented them. Using any chemical in this way constitutes drug use and is no more legal, especially for fish released into the wild, than is the use of unapproved anesthetics or antibiotics.

Disposal of Medical Waste

Scalpel blades, hypodermic needles, and syringes are considered medical waste, and their disposal is regulated by state laws that may vary. Such waste should never be placed in domestic garbage for disposal. It is in the best interests of wildlife biologists and veterinarians to prevent further expansion of laws restricting disposal of medical wastes by properly disposing of such materials. Medical waste should be placed in "sharps" containers that are correctly labeled with the biohazard symbol. These containers should be returned to the laboratory from the field and disposed of in a legal fashion (usually by incineration in an approved incinerator). Commercial services can handle the legal disposal of such wastes.

Recovery and Release

An implanted fish should not be released into natural waters until it has fully recovered from the effects of anesthesia. Fish should be allowed to recover from anesthesia in a protected environment comprising large amounts of high-quality, well-oxygenated water of a temperature suitable for the species of fish and resembling the environment to which they will be returned. The water should be changed frequently to maintain its temperature and quality and to avoid the accumulation of metabolites, including alarm substances, released by other fish. As a rule, it is better to release the fish as soon as they are fully recovered than it is to hold them for more than a few hours.

Anesthesia and surgery produce profound physiological changes that affect body chemistry, hematology, and endocrine response (Houston et al. 1969, 1971; Randall 1962; Randall et al. 1965; Shelton and Randall 1962; Wedemeyer 1969, 1970). Although clearance of the anesthetic from the blood may occur rapidly (Houston et al. 1971), significant tissue residues can be detected for several hours (Walker and Schoettger 1967). Surgery itself adds a short-term stress in addition to the stress from anesthesia. Chinook salmon smolts surgically implanted with transmitters had elevated plasma cortisol levels after 24 hr compared with control fish that were handled and anesthetized, but the difference had disappeared 7 days after surgery (Jepsen et al. 2001). Implanted fish are typically released into the wild very soon after surgery, and the residual stresses from handling, anesthesia, and surgery may continue to have an adverse physiological effect.

Reporting of Methods and Results

The use of live animals in research involving an invasive procedure should be performed only when there is a question of sufficient importance to make both the expense of the project and the potential adverse effects on the fish worthwhile. As part of that justification, the reports of the study should be published in the primary scientific literature. Results should be published even if the experiment revealed problems with the implantation of transmitters, which made the experiment moot. Publication of such results in the scientific literature may assist other researchers in planning and performing their own experiments on the same or similar species of fish.

The published results of telemetry studies should include a justification for the selection of implantation as a method for the attachment of transmitters to fish. In addition, authors should present data to confirm that the techniques used did not produce adverse effects on the host fish and will not affect the quality and reliability of the resulting data.

Possible Exceptions

Exceptions to the standards of anesthesia and surgery presented in this review should be rare. Inconvenience or a lack of funding, knowledge, equipment, time, or interest is never a valid reason for performing anesthesia or surgery in less than an optimal manner. Most of the requirements for adequate anesthesia and surgery on fish are matters of preparation before work is done in the field, and there should be few acceptable excuses for inadequate preparation. Acceptable reasons for compromising the standards of anesthesia and surgery may relate to human and animal safety and the possibility of exceeding practicality. For example, a transmitter is surgically implanted into a large shark by inverting the shark in the water and operating while the fish is still in the water, with its abdomen at the water's surface (Holland et al. 1999). Seawater almost inevitably washes into the surgical incision. For large sharks, the impracticality of hoisting them from the water and the obvious safety problems for both the fish and the humans involved require some form of compromise.

Starr et al. (2000) used a novel technique while surgically implanting transmitters in rockfish (*Sebastes* spp.) to reduce temperature shock and decompression barotrauma to the fish that had been hooked at 100 to 200 m. Instead of bringing the fish all the way to the surface, scuba divers performed the surgery under water at 20 m, below the thermocline. This technique avoided the damage from swimbladders that overinflated when the fish were brought to the surface and removed from the water. However, the pressure differential between the 100 to 200 m capture depth and the 20 m surgical depth meant that the swim bladders of an unstated number of fish still had to be decompressed before surgery could begin, and therefore the true value of this procedure must be questioned. Other studies have used deflation of swim bladders of fish brought to the surface (Bruesewitz et al. 1993; Collins et al. 1999; Keniry et al. 1996; Lee 1992). Starr et al. (2000) did not present experimental data documenting that swim bladder deflation of all of their fish would not work. Thus, the potential advantage of performing the surgery under water must be weighed against the use of nonsterile instruments and transmitters, and opening the coelom of the fish to ocean water.

If compromises are made in the short term, researchers should continually seek ways of improving the quality of the surgeries they perform. Scientists should present full justifications to the appropriate IACUC, detailing alternatives that were considered and the reasons for their rejection. IACUCs that approve exceptions in technique for a specific project should avoid making such exceptions permanent.

Conclusion

Fish are exempted from coverage by the Animal Welfare Act; however, legal requirements such as the IRAC Principles and policies such as those promulgated by the US Public Health Service mean that projects involving free-ranging fish are being increasingly reviewed by IACUCs. Surgical implantation of transmitters into fish is a valuable technique for research on free-ranging fish. Standards of care have not been established for the technical procedures for anesthesia and surgery used for the implantation of transmitters. IACUCs therefore lack guidance for evaluating such projects. There are no reasons that most standards of care for the performance of anesthesia and surgery on captive animals cannot be applied to surgery and anesthesia in the field on free-ranging fish. It is reasonable to expect that aseptic technique will include the use of sterile (not disinfected) instruments and transmitters and that surgeons will wear sterile gloves.

Nevertheless, IACUCs must accept that the anesthesia and surgery performed on fish in field situations may present real limitations and force compromise. By definition, surgeries in the field cannot be performed in dedicated surgical suites. It may not be practical to remove very large, dangerous fish from water to perform the surgeries, yet exceptions should be relatively few and should be well documented to the IACUC by scientists. Besides concern for the fish being used, the overriding goal of both scientists and IACUCs should be to ensure that the procedures used to implant transmitters into fish minimize iatrogenic adverse effects that might lead to inaccurate conclusions.

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